

Rat 2891-2894

(Book 14)

McAb to x1053

human iNOS CT: CR(NH)(OM) SLEMSAL / started 4/20/93
1st time in the -20°C

① 1st immunization IP \geq FCA.

Note: O. & w. aqueous
+ O. & w. TCA

1. One factor, then one / set.

② 2nd immunization IP Ⓛ FCA 5-12-93

③ 3rd IP ē FCA 6-3-93

④ Test bleed from ~~left~~ Tail 6-9-93

⑤ 4th JP C FCA JC/AF 6-22-93

⑥ Rats # 2893 + 2894 were boosted IP + intraperitoneal
~~05/22/2003 (1053/1185 in conj. #2)~~

0.5 ml \Rightarrow 200 μ gm NaOS3/Phyo. conjugate #2
per ml; sterile filtered & dissolved in PBS

(7) r 2893 - Term. A/s 7-3-93

⑧ 12894 - Term. A/S 7-6-93

⑨ 5th IP ∈ FCA RW/SC

8-10-93

(15) Test Bleed from Tail r2891 + r2692 BW 9/2/93

⑪ r2891 and r2892 boosted 1f + missing: 1V, final best prior twikf 9/14/93
to fusion

ESSAY TEAM

Plate # 1 ND53 rats Date 6 / 18 / 93 Filter nm
Operator

Blank

Dec. 3 4

2008 '214

17102,400

EASY BEAM

Plate # ND53 rats Date 6 / 18 / 93 Filter _____ nm
 Operator _____ Comment _____

	1	2	3	4	5	6	7	8	9	10	11	12		
r2891	A	+0.071	+1.526	+1.470	+1.289	+1.102	+0.896	+0.614	+0.351	+0.238	+0.122	+0.114	+0.081	A IgG
r2891	B	-0.005	+0.335	+0.170	+0.093	+0.048	+0.025	+0.011	+0.000	+0.000	-0.009	-0.009	-0.011	B IgM
r2892	C	+0.078	+1.424	+1.396	+1.218	+1.034	+0.847	+0.594	+0.407	+0.285	+0.175	+0.101	+0.094	C IgG
r2892	D	+0.000	+1.325	+0.956	+0.585	+0.338	+0.186	+0.089	+0.043	+0.014	+0.000	+0.000	+0.000	D IgM
r2893	E	+0.582	+1.822	+1.841	+1.763	+1.813	+1.771	+1.640	+1.531	+1.380	+1.218	+0.896	+0.623	E IgG
r2893	F	+0.000	+1.779	+1.377	+0.765	+0.372	+0.194	+0.094	+0.049	+0.020	+0.005	+0.000	+0.000	F IgM
r2894	G	+0.159	+1.671	+1.623	+1.417	+1.194	+0.968	+0.672	+0.426	+0.285	+0.205	+0.200	+0.124	G IgG
r2894	H	+0.000	+0.527	+0.291	+0.173	+0.089	+0.042	+0.019	+0.006	+0.000	+0.000	+0.000	+0.000	H IgM

1	2	3	4	5	6	7	8	9	10	11	12
blank	1 → 100	1 → 200	1 → 400	1 → 800	1 → 1,000	1 → 3,100	1 → 6,100	1 → 12,100	1 → 25,100	1 → 51,200	1 → 102,400

NO 53 FUSION

7/6/93

Y3.1 12XT-75

Harvested as separate groups floating and attached cells

Cell Counts and Viability: Estimates

1. Floating $94/95 \times 10^4 \times 10^4 = 9.4 \times 10^8 / \text{ml}$ $9.4 \times 10^8 / \text{ml} (36 \text{ ml})$ $\approx 3.38 \times 10^8 \text{ cells total - viable}$ $94/95 \text{ alive} = 98.9\% \text{ viability}$

2. Attached

 $113 \times 10^6 \text{ cells (124)} = 113 \times 10^8 \text{ cells}$

C. Viability

113 alive / 115 total

(Many still attached to bottom of flask, difficult to remove)

Splenocytes (Rat)

Spleen #1 (Rat #2873) :

Pool A = 261 @ 21 ml

Pool B = 317 @ 21 ml $578 \times 10^4 \text{ cells} = 5.78 \times 10^8 \text{ cells (21 ml)}$
 $= 1.67 \times 10^8 \text{ cells}$

Spleen #2 (Rat 2874) :

 $259 \times 10^4 \times 45 = 1.16 \times 10^8 \text{ cells}$
+ $\sim 25\% \text{ extra from } \frac{30 \times 10^8 \text{ cells}}{1.46 \times 10^8 \text{ cells}}$ Total splenocytes = $1.67 \times 10^8 + 1.46 \times 10^8 \approx 3 \times 10^8 \text{ splenocytes total}$ Ratio of splenocytes: Y3.1 = 2:1 \therefore use:

- 1) 3×10^8 splenocytes
- 2) 1.5×10^8 Y3.1

Y3.1 2nd count after spun down 2x: $514 \times 10^4 \times 44 \text{ ml} = 2.6 \times 10^8 \text{ Y3 total}$ $1.5 \times 10^8 \text{ cells} = 25 \text{ ml stock}$

SCREENING Master Plates
NOS3 fusion

EASY BEAM

Plate # 1 Date 8, 5, 93 Filter 492 nm
Operator DF/KF Comment NOS3 AT 100ng/well

	1	2	3	4	5	6	7	8	9	10	11	12
A	+0.000	+0.000	+0.000	+0.029	+0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000
B	+0.008	+0.000	+0.026	+0.021	+0.013	+0.000	+0.000	+0.000	+0.006	+0.006	+0.006	+0.000
C	+0.007	+0.007	+0.007	+0.007	+0.007	+0.025	+0.009	+0.009	+0.014	+0.007	+0.000	+0.007
D	+0.000	+0.000	+0.000	+0.000	+0.014	+0.022	+0.006	+0.006	+0.015	+0.000	+0.008	+0.008
E	+0.000	+0.000	+0.000	+0.000	-0.005	+0.022	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000
F	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000
G	+0.000	+0.000	+0.000	+0.000	+0.005	+0.007	+0.007	+0.007	+0.013	+0.011	+0.006	+0.000
H	+0.000	+0.000	+0.000	+0.000	+0.006	+0.006	+0.010	+0.010	+0.005	+0.008	+0.006	+0.006

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # 2 Date 8/5/93 Filter 492 nm
 Operator DF/KF Comment NAS3 @ 100ug/well

	1	2	3	4	5	6	7	8	9	10	11	12
A	+0.000	+0.000	+0.009	+0.009	+0.009	+0.011	+0.009	+0.000	-0.010	-0.006	-0.010	-0.005
B	-0.012	-0.012	-0.007	-0.012	-0.010	-0.010	-0.006	-0.010	-0.010	+0.000	-0.005	-0.010
C	-0.017	-0.011	-0.007	+0.000	-0.011	-0.011	-0.011	-0.011	-0.011	-0.011	-0.009	-0.009
D	-0.009	-0.012	-0.007	-0.013	-0.008	-0.006	-0.016	-0.016	-0.016	-0.009	-0.011	-0.009
E	-0.012	-0.012	-0.010	+0.000	+0.009	+0.014	-0.014	-0.019	-0.013	-0.007	-0.013	+0.000
F	+0.012	+0.000	-0.016	-0.016	-0.016	-0.013	-0.015	-0.015	-0.015	-0.013	-0.013	-0.018
G	-0.014	+0.008	-0.013	-0.017	-0.013	-0.010	-0.013	-0.016	-0.018	-0.013	-0.011	-0.013
H	-0.022	-0.016	-0.014	-0.014	-0.014	-0.005	-0.018	-0.018	-0.018	-0.013	-0.013	-0.013

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # 3 Date 8, 5, 93 Filter 492 nm
 Operator DF/KF Comment N053 @ 100 ng/well

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.005	+0.009	+0.007	+0.012	+0.008	+0.008	+0.011	+0.007	+0.011	A
B	+0.011	+0.009	+0.009	+0.009	+0.009	+0.009	+0.009	+0.011	+0.011	+0.015	+0.015	+0.008	B
C	+0.006	+0.010	+0.010	+0.008	+0.008	+0.011	+0.009	+0.011	+0.009	+0.009	+0.013	+0.007	C
D	+0.007	+0.009	+0.009	+0.009	+0.000	+0.025	+0.010	+0.012	+0.007	+0.007	+0.010	+0.010	D
E	+0.008	+0.006	+0.000	+0.000	+0.019	+0.031	+0.013	+0.011	+0.020	+0.007	+0.011	+0.011	E
F	+0.011	+0.011	+0.011	+0.007	+0.010	+0.040	+0.020	+0.013	+0.016	+0.016	+0.012	+0.014	F
G	+0.020	+0.006	+0.029	+0.022	+0.017	+0.007	+0.007	+0.015	+0.013	+0.009	+0.013	+0.013	G
H	+0.006	+0.006	+0.008	+0.036	+0.010	+0.000	+0.000	+0.008	+0.000	+0.009	+0.018	+0.008	H

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 # 4 Date 8 / 5 / 93 Filter _____ nm
 Operator _____ Comment N053 screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.010	+0.012	+0.005	+0.009	+0.009	+0.000	-0.010	+0.012	+0.000	+0.005	A
B	+0.000	+0.007	+0.007	+0.033	+0.024	+0.027	+0.016	+0.010	+0.000	+0.000	+0.000	+0.000	B
C	+0.012	-0.006	+0.014	+0.010	+0.000	+0.018	+0.000	+0.006	-0.007	+0.000	-0.006	+0.000	C
D	+0.012	+0.000	+0.017	+0.000	+0.000	+0.000	+0.005	+0.000	+0.010	-0.005	+0.000	+0.000	D
E	+0.014	+0.010	+0.007	+0.018	+0.029	+0.007	+0.000	+0.005	+0.020	+0.000	-0.006	+0.005	E
F	+0.017	+0.006	+0.006	+0.017	+0.017	+0.038	+0.010	+0.012	+0.030	+0.005	+0.000	+0.009	F
G	+0.016	+0.025	+0.010	+0.017	+0.000	+0.024	+0.000	+0.000	+0.000	+0.009	+0.000	+0.012	G
H	+0.017	+0.020	+0.010	+0.022	+0.000	+0.010	+0.014	+0.011	+0.000	+0.000	+0.010	+0.013	H

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 # 5 Date 8 / 15 / 93 Filter _____ nm
 Operator _____ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.007	+0.000	-0.006	+0.010	+0.000	+0.000	+0.029	-0.005	+0.000	+0.000	-0.007	A
B	+0.024	+0.028	+0.013	+0.000	+0.006	-0.010	+0.000	+0.000	-0.010	+0.000	+0.000	+0.000	B
C	+0.000	+0.000	+0.014	-0.006	+0.000	+0.009	-0.006	-0.006	+0.007	-0.005	+0.000	+0.000	C
D	+0.022	+0.006	+0.000	+0.012	+0.010	+0.000	-0.005	+0.011	-0.008	+0.005	+0.012	+0.000	D
E	+0.005	+0.027	+0.008	+0.010	+0.020	+0.022	+0.012	+0.000	+0.000	+0.012	+0.029	+0.000	E
F	+0.006	+0.010	+0.006	+0.008	+0.013	+0.025	+0.016	+0.000	+0.008	+0.000	+0.000	+0.006	F
G	+0.000	+0.005	+0.009	+0.005	+0.022	+0.016	+0.010	+0.000	+0.009	+0.000	+0.000	+0.010	G
H	+0.000	+0.029	+0.037	+0.034	+0.016	+0.025	+0.000	+0.008	+0.020	+0.005	+0.000	+0.000	H

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 # 6 Date 8 / 5 / 93 Filter _____ nm
 Operator _____ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	+0.008	+0.006	+0.000	+0.007	+0.000	+0.129	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000
B	+0.006	+0.015	+0.015	+0.000	+0.000	-0.009	+0.011	-0.008	-0.008	-0.008	+0.000	+0.014
C	+0.012	+0.008	+0.033	+0.000	+0.008	+0.000	+0.000	-0.005	+0.006	-0.008	+0.000	+0.010
D	+0.007	+0.007	+0.000	+0.010	+0.000	+0.000	+0.000	-0.005	-0.005	+0.000	+0.000	+0.000
E	+0.000	+0.005	+0.017	+0.005	+0.016	+0.000	+0.012	+0.005	+0.014	+0.000	+0.000	+0.000
F	+0.014	+0.012	+0.021	+0.027	+0.018	+0.018	+0.013	+0.000	+0.013	+0.000	+0.000	+0.006
G	+0.014	-0.005	+0.018	+0.008	+0.000	+0.014	+0.033	+0.000	+0.005	+0.008	+0.000	+0.000
H	<u>+0.053</u>	+0.006	+0.009	+0.019	+0.040	+0.013	+0.000	+0.000	-0.005	+0.006	+0.000	+0.000

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 #7 Date 8 / 15 / 93 Filter _____ nm
 Operator _____ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.019	+0.008	+0.016	+0.009	+0.000	+0.013	+0.017	+0.007	+0.005	+0.007	+0.009	+0.000	A
B	+0.000	+0.026	+0.019	+0.014	+0.000	+0.029	+0.011	+0.007	+0.011	+0.013	+0.009	+0.006	B
C	+0.009	+0.024	+0.006	+0.008	+0.010	+0.012	+0.017	+0.024	+0.008	+0.008	+0.026	+0.008	C
D	+0.012	+0.016	+0.019	+0.019	+0.038	+0.026	+0.040	+0.012	+0.016	+0.008	+0.015	+0.015	D
E	+0.008	+0.010	+0.013	+0.010	+0.016	+0.016	+0.018	+0.016	+0.048	+0.030	+0.025	+0.010	E
F	+0.035	+0.019	+0.009	+0.005	+0.026	+0.028	+0.021	+0.019	+0.019	+0.021	+0.012	+0.016	F
G	+0.000	+0.016	+0.032	+0.008	+0.023	+0.031	+0.027	+0.030	+0.018	+0.008	+0.027	+0.016	G
H	+0.026	+0.009	+0.012	+0.012	+0.012	+0.024	+0.033	+0.025	+0.014	+0.017	+0.020	+0.031	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053 #8 Date 8 / 5 / 92 Filter _____ nm
 Operator Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.013	-0.008	+0.008	+0.005	+0.000	+0.026	+0.000	+0.000	+0.000	+0.000	+0.000	-0.011	A
B	+0.019	-0.018	+0.000	+0.025	+0.011	+0.011	+0.000	+0.000	+0.019	-0.008	-0.008	+0.000	B
C	+0.015	-0.011	+0.007	-0.009	+0.000	+0.000	+0.043	+0.000	+0.000	+0.000	+0.000	-0.005	C
D	+0.017	+0.000	-0.008	+0.019	+0.016	-0.007	+0.005	+0.009	+0.013	+0.017	-0.008	-0.005	D
E	+0.009	+0.000	+0.010	+0.000	+0.023	+0.000	+0.000	+0.000	+0.000	-0.008	-0.005	+0.000	E
F	+0.000	+0.000	+0.019	+0.013	+0.006	+0.012	-0.006	+0.011	+0.009	+0.000	+0.000	-0.010	F
G	+0.074	+0.000	+0.006	+0.000	+0.010	-0.005	+0.007	+0.000	+0.000	+0.000	-0.014	+0.027	G
H	+0.023	+0.033	+0.010	+0.005	+0.000	+0.000	+0.010	+0.007	+0.005	+0.000	-0.010	-0.008	H

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # NO53 # 9 Date 8 / 5 / 93 Filter _____ nm
 Operator _____ Comment NO53 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+	+	+	+	+	+	+	+	+	+	+	+	A
A	+0.038	-0.007	+0.008	+0.000	+0.000	+0.010	-0.008	-0.008	+0.000	+0.000	-0.008	+0.000	
B	+	+	+	+	+	+	+	+	+	+	+	+	B
B	+0.007	+0.013	+0.009	+0.017	+0.015	-0.006	-0.006	+0.000	+0.008	-0.010	-0.010	+0.000	
C	+	+	+	+	+	+	+	+	+	+	+	+	C
C	+0.007	+0.019	+0.010	+0.006	+0.021	+0.000	+0.000	+0.000	+0.000	-0.012	-0.010	-0.010	
D	+	+	+	+	+	+	+	+	+	+	+	+	D
D	+0.005	+0.009	+0.019	+0.011	+0.016	+0.000	+0.006	+0.049	+0.006	+0.000	-0.005	+0.000	
E	+	+	+	+	+	+	+	+	+	+	+	+	E
E	+0.000	+0.015	+0.021	+0.033	+0.005	+0.005	+0.005	+0.005	+0.024	-0.011	+0.011	-0.010	
F	+	+	+	+	+	+	+	+	+	+	+	+	F
F	+0.062	+0.023	+0.031	+0.046	+0.017	+0.045	+0.055	+0.000	+0.000	-0.008	-0.006	+0.000	
G	+	+	+	+	+	+	+	+	+	+	+	+	G
G	+0.051	+0.016	+0.016	+0.021	+0.013	+0.013	+0.000	+0.008	+0.013	+0.000	-0.010	+0.008	
H	+	+	+	+	+	+	+	+	+	+	+	+	H
H	+0.008	+0.000	+0.022	+0.018	+0.007	+0.043	+0.026	+0.011	+0.018	+0.005	+0.020	+0.000	

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 #11 Date 6 / 5 / 93 Filter _____ nm
 Operator _____ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	+0.012	-0.007	+0.000	+0.000	+0.000	-0.007	+0.016	+0.000	-0.006	-0.010	-0.005	+0.017
B	+0.000	+0.000	+0.009	+0.016	+0.014	+0.000	+0.031	-0.008	-0.008	+0.000	+0.015	+0.000
C	+0.000	+0.027	+0.000	+0.000	+0.016	+0.024	+0.054	-0.005	+0.006	-0.007	+0.007	+0.007
D	+0.009	+0.012	+0.000	+0.007	+0.027	+0.010	+0.019	+0.006	-0.017	-0.017	-0.010	-0.005
E	+0.030	+0.000	+0.009	+0.006	+0.006	+0.000	+0.034	+0.005	+0.065	-0.015	+0.005	+0.053
F	+0.006	+0.000	+0.000	+0.019	+0.019	+0.038	+0.065	+0.000	-0.014	-0.007	+0.000	-0.012
G	+0.009	+0.016	-0.010	+0.000	+0.013	-0.009	+0.007	-0.009	+0.008	-0.016	+0.000	+0.000
H	+0.000	+0.026	+0.000	+0.000	-0.006	-0.006	+0.019	+0.000	+0.000	-0.009	+0.000	-0.010

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 - JgG Date 6 / 11 / 93 Filter _____ nm
 Operator _____ Comment N053 Titration Assay

	1	2	3	4	5	6	7	8	9	10	11	12	Blank
	64.6	64.1	86.1	9F1	9F7	96.1	1LC7	NE12	11F3				
A	+0.026	+0.017	+0.000	+0.034	+0.014	+0.007	+0.028	+0.000	+0.008	+0.038	+0.040	+0.040	A 1:2
B	+0.032	+0.013	+0.022	+0.006	+0.000	+0.009	+0.005	+0.005	+0.000	+0.036	+0.044	+0.044	B 1:4
C	+0.013	-0.008	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.054	+0.038	+0.052	C 1:8
D	+0.015	+0.000	+0.009	+0.005	+0.000	+0.017	+0.005	+0.046	+0.012	+0.043	+0.038	+0.065	D 1:16
E	+0.010	+0.000	+0.018	+0.020	+0.006	+0.000	+0.018	+0.000	+0.012	+0.054	+0.062	+0.049	E 1:32
F	+0.008	+0.010	+0.007	+0.007	+0.005	+0.000	+0.000	+0.000	+0.047	+0.056	+0.042	+0.042	F 1:64
G	+0.000	+0.011	+0.000	+0.014	+0.018	+0.014	+0.000	-0.009	+0.011	+0.079	+0.046	+0.042	G 1:128
H	+0.009	+0.005	+0.000	+0.012	+0.035	+0.018	+0.020	+0.007	+0.014	+0.048	+0.037	+0.046	H 1:256
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # NO53 - IgM Date 8 / 11 / 93 Filter _____ nm
 Operator _____ Comment NO53 Titration Assay

	Blank											
	1	2	3	4	5	6	7	8	9	10	11	12
A	+0.000	+0.000	+0.015	+0.011	+0.020	+0.000	+0.000	+0.036	-0.006	+0.000	+0.000	+0.000
B	+0.000	+0.000	+0.009	+0.000	+0.009	+0.000	+0.000	+0.019	+0.000	+0.000	+0.000	+0.000
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	-0.005	+0.000	+0.000
D	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000
E	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000
F	+0.000	+0.000	+0.000	+0.000	+0.007	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000
H	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000
	1	2	3	4	5	6	7	8	9	10	11	12

NO53 FUSION

Y31

Viable Cell Count \rightarrow Y31 harvested by drawing med a into pipet then dislodging cells adherent to flask w/ a stream of media

$$\begin{aligned}
 \text{Pool A} & 305 \times 10^4 \text{ cells/ml (40ml)} = 8.2 \times 10^7 \text{ cells} \\
 \text{B} & 186 \times 10^4 \text{ cells/ml (45ml)} = 8.3 \times 10^7 \text{ cells} \\
 \text{C} & 330 \times 10^4 \text{ cells/ml (35ml)} = 11.6 \times 10^7 \text{ cells} \\
 \text{D} & 304 \times 10^4 \text{ cells/ml (35ml)} = 10.3 \times 10^7 \text{ cells} \\
 \text{E} & 8.75 \times 10^7 \text{ cells}
 \end{aligned}$$

Total # Y31 = 4.75×10^8 cells in 10 ml \Rightarrow 95.8% avg viability

Splenocytes

$$\begin{aligned}
 \text{Count A} & 210 \times 10^4 \text{ cells (10 fold dilution) (25ml)} = 5.25 \times 10^8 \text{ splenocytes} \\
 \text{B} & 164 \times 10^4 \text{ (10 fold dilution) (25ml)} = 4.1 \times 10^8 \text{ splenocytes}
 \end{aligned}$$

$$\bar{X} = \frac{4.6}{2} \times 10^8 \text{ splenocytes}$$

\Rightarrow At 2:1 ratio of splen. : myeloma (Y31)

$$\Rightarrow \text{NECA} \approx 2.05 \times 10^8 \text{ Y31} \Rightarrow 4.1 \text{ ml} \Rightarrow \text{vol} \approx 5 \text{ ml}$$

[Monday 8/20/93]

Y31/2-Ag $\frac{1}{2}$

2xT75 plated off cells from back of flask then counted

$$\text{Flask B} : 105 \times 10^4 \text{ cells/ml (1 ml)} = 1.05 \times 10^7 \text{ cells}$$

$$\text{Flask C} : 106 \times 10^4 \text{ cells/ml (1 ml)} = 1.06 \times 10^7 \text{ cells}$$

$$\bar{X} \text{ viability} = 92.5\%$$

2xT75 added 1 ml media

Media + FBS + L-FCG \Rightarrow 1.05

Y31

2xT75 added 1 ml media to each flask for 1 week (two wks)

EASY BEAM

Plate # NO53 rats Date 9 / 3 / 93 Filter nm
 Operator Comment NO53 Rats - 2nd Test Bleed
Titration Assay - kF

	1	2	3	4	5	6	7	8	9	10	11	12	
r2891	A	+0.057	+1.564	+1.601	+1.268	+1.050	+0.643	+0.511	+0.329	+0.245	+0.173	+0.117	+0.109
r2892	B	+0.079	+1.891	+1.560	+1.740	+1.780	+1.653	+1.491	+1.246	+0.957	+0.675	+0.403	+0.322
r2891	C	+0.012	+1.085	+0.802	+0.529	+0.338	+0.192	+0.108	+0.062	+0.040	+0.029	+0.020	+0.017
r2892	D	+0.008	+1.936	+1.428	+0.807	+0.426	+0.244	+0.130	+0.074	+0.045	+0.029	+0.021	+0.017
	E	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	E
	F	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	F
	G	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	G
	H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
Blank		1→100	1→200	1→400	1→800	1→1600	1→3200	1→6400	1→12800	1→25600	1→51200	1→102400	

EASY BEAM

Plate # NO53#1 Date 10 / 12 / 93 Filter _____ nm
 Operator _____ Comment ELISA screen on NO53

	1	2	3	4	5	6	7	8	9	10	11	12
A	+0.010	+0.047	+0.043	+0.007	+0.000	0.007	-0.009	+0.000	-0.006	+0.000	+0.000	+0.013
B	+0.010	+0.022	+0.041	+0.000	+0.015	+0.015	+0.018	+0.046	+0.022	+0.036	+0.046	+0.000
C	+0.007	+0.092	+0.034	+0.000	+0.000	+0.019	+0.037	+0.009	+0.035	+0.000	+0.031	+0.031
D	+0.039	+0.010	+0.071	+0.014	+0.017	+0.037	+0.046	+0.009	+0.046	+0.000	+0.031	+0.050
E	+0.035	+0.009	+0.052	+0.027	+0.005	+0.021	+0.000	+0.007	+0.000	+0.000	+0.016	+0.365
F	+0.026	+0.009	+0.014	+0.021	+0.035	+0.022	+0.000	+0.036	+0.014	+0.000	+0.031	+0.005
G	+0.000	+0.018	+0.008	+0.017	+0.000	+0.015	+0.007	+0.013	+0.005	+0.012	-0.013	+0.020
H	+0.000	+0.015	+0.058	+0.064	+0.009	+0.041	+0.012	+0.011	+0.057	+0.000	+0.033	+0.044

1 2 3 4 5 6 7 8 9 10 11 12

1E12

EASY BEAM

Plate # NO53 #2 Date 10 / 12 / 93 Filter _____ nm
Operator Comment NO53 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	+0.025	+0.000	+0.000	+0.019	+0.000	0.009	+0.000	+0.048	+0.006	+0.000	+0.007	+0.000
B	-0.011	+0.000	+0.055	+0.005	+0.000	+0.035	-0.006	+0.020	+0.000	+0.036	+0.042	+0.005
C	+0.014	+0.000	+0.000	+0.006	+0.026	+0.006	+0.017	+0.000	+0.000	+0.009	+0.007	+0.000
D	+0.017	+0.042	+0.026	+0.011	+0.008	+0.014	+0.017	+0.000	+0.008	+0.005	+0.091	-0.007
E	+0.032	+0.012	+0.012	+0.012	+0.012	+0.010	+0.305	-0.470	+0.015	+0.000	+0.009	+0.041
F	-0.005	+0.012	+0.007	+0.018	+0.000	+0.026	-0.005	+0.011	+0.000	+0.013	+0.018	+0.000
G	-0.008	-0.005	+0.028	+0.016	+0.016	+0.300	+0.000	+0.000	+0.000	-0.012	+0.020	-0.006
H	-0.376	+0.027	+0.000	+0.012	+0.008	+0.000	+0.023	+0.000	+0.000	+0.000	+0.000	+0.061
	1	2	3	4	5	6	7	8	9	10	11	12

2 E 8

EASY BEAM

Plate # N053 #3 Date 10 / 12 / 93 Filter _____ nm
 Operator _____ Comment _____

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.016	-0.010	-0.005	+0.023	+0.016	+0.000	+0.033	+0.012	+0.000	+0.000	+0.007	A
B	+0.005	+0.005	+0.000	-0.011	-0.011	-0.011	-0.015	+0.000	+0.010	+0.005	+0.012	+0.014	B
C	+0.006	+0.000	+0.000	-0.005	-0.008	+0.000	+0.007	+0.000	-0.007	+0.011	-0.015	-0.006	C
D	+0.060	+0.017	+0.009	-0.009	+0.037	+0.000	-0.006	+0.000	+0.006	-0.011	+0.000	+0.000	D
E	+0.010	+0.033	+0.000	-0.006	+0.010	-0.011	+0.000	-0.009	+0.000	+0.016	-0.009	-0.005	E
F	+0.000	+0.014	+0.000	+0.000	+0.005	+0.007	+0.000	+0.014	+0.016	+0.019	+0.000	+0.006	F
G	+0.013	+0.013	+0.017	-0.006	-0.006	+0.000	+0.000	+0.000	+0.019	+0.051	+0.013	+0.013	G
H	+0.021	+0.028	+0.008	+0.000	+0.000	+0.006	+0.000	+0.000	+0.032	+0.000	+0.000	+0.047	H

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # NO53 #4 Date 10 / 12 / 93 Filter _____ nm
 Operator Comment NO53 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	+0.000	+0.000	+0.010	+0.012	+0.005	+0.009	+0.009	+0.000	-0.010	+0.012	+0.000	+0.005
B	+0.000	+0.007	+0.007	+0.033	+0.024	+0.027	+0.016	+0.010	+0.000	+0.000	+0.000	+0.000
C	+0.012	-0.006	+0.014	+0.010	+0.000	+0.018	+0.000	+0.006	-0.007	+0.000	-0.006	+0.000
D	+0.012	+0.000	+0.017	+0.000	+0.000	+0.000	+0.005	+0.000	+0.010	-0.005	+0.000	+0.000
E	+0.014	+0.010	+0.007	+0.018	+0.029	+0.007	+0.000	+0.005	+0.020	+0.000	-0.006	+0.005
F	+0.017	+0.006	+0.006	+0.017	+0.017	+0.038	+0.010	+0.012	+0.030	+0.005	+0.000	+0.009
G	+0.016	-0.025	-0.010	+0.017	+0.000	+0.024	+0.000	+0.000	+0.009	+0.000	+0.012	+0.000
H	-0.017	+0.020	-0.010	-0.022	+0.000	+0.010	+0.014	+0.011	+0.000	+0.000	+0.010	+0.013

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 # 5 Date 10 / 12 / 93 Filter _____ nm
 Operator Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.007	+0.000	-0.006	+0.010	+0.000	+0.000	+0.029	-0.005	+0.000	+0.000	-0.007	A
B	+0.024	+0.028	+0.013	+0.000	+0.006	-0.010	+0.000	+0.000	-0.010	+0.000	+0.000	+0.000	B
C	+0.000	+0.000	+0.014	-0.006	+0.000	+0.009	-0.006	-0.006	+0.007	-0.005	+0.000	+0.000	C
D	+0.022	+0.005	+0.000	+0.012	+0.010	+0.000	-0.005	+0.011	-0.008	+0.005	+0.012	+0.000	D
E	+0.005	+0.027	+0.008	+0.010	+0.020	+0.022	+0.012	+0.000	+0.000	+0.012	+0.029	+0.000	E
F	+0.006	+0.010	+0.006	+0.008	+0.013	+0.025	+0.016	+0.000	+0.008	+0.000	+0.000	+0.006	F
G	+0.000	+0.005	+0.009	+0.005	+0.022	+0.016	+0.010	+0.000	+0.009	+0.000	+0.000	+0.010	G
H	+0.000	+0.029	+0.037	+0.034	+0.016	+0.025	+0.000	+0.008	+0.020	+0.005	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053 # 6 Date 10 / 12 / 93 Filter _____ nm
 Operator _____ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.015	+0.013	+0.025	+0.035	+0.037	+0.008	+0.008	+0.006	+0.000	+0.015	+0.011	A
B	-0.00	+0.000	+0.021	+0.021	+0.000	+0.017	+0.012	+0.077	+0.007	-0.006	+0.022	+0.012	B
C	+0.018	+0.042	+0.016	+0.023	+0.007	+0.007	+0.000	+0.000	+0.016	-0.008	+0.033	+0.048	C
D	+0.013	+0.042	+0.047	+0.016	+0.024	+0.016	+0.037	+0.049	+0.039	+0.000	+0.036	+0.048	D
E	+0.020	+0.037	+0.020	+0.020	+0.000	+0.028	+0.025	+0.000	+0.017	+0.010	+0.006	+0.026	E
F	+0.000	+0.017	+0.024	+0.015	+0.007	+0.041	+0.015	+0.000	+0.032	+0.007	+0.005	+0.041	F
G	+0.027	+0.030	+0.030	+0.015	+0.018	+0.050	+0.010	-0.008	-0.008	+0.000	+0.044	+0.044	G
H	+0.000	+0.011	+0.000	+0.000	+0.011	+0.007	+0.000	-0.010	+0.283	+0.090	+0.061	+0.027	H
	1	2	3	4	5	6	7	8	9	10	11	12	

6 H 9

EASY BEAM

Plate # N053 #7 Date 10 / 12 / 93 Filter _____ nm
 Operator _____ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	+0.019	+0.008	+0.016	+0.009	+0.000	+0.013	+0.017	+0.007	+0.005	+0.007	+0.009	+0.000
B	+0.000	+0.026	+0.019	+0.014	+0.000	+0.029	+0.011	+0.007	+0.011	+0.013	+0.009	+0.006
C	+0.009	+0.024	+0.006	+0.008	+0.010	+0.012	+0.017	+0.024	+0.008	+0.008	+0.026	+0.008
D	+0.012	+0.016	+0.019	+0.019	+0.038	+0.026	+0.040	+0.012	+0.016	+0.008	+0.015	+0.015
E	+0.008	+0.010	+0.013	+0.010	+0.016	+0.016	+0.018	+0.016	+0.048	+0.030	+0.025	+0.010
F	+0.035	+0.019	+0.009	+0.005	+0.026	+0.028	+0.021	+0.019	+0.019	+0.021	+0.012	+0.016
G	+0.000	+0.016	+0.032	+0.008	+0.023	+0.031	+0.027	+0.030	+0.018	+0.008	+0.027	+0.016
H	+0.026	+0.009	+0.012	+0.012	+0.012	+0.024	+0.033	+0.025	+0.014	+0.017	+0.020	+0.031

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 #B Date 10 / 12 / 92 Filter _____ nm
 Operator Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.013	-0.008	+0.008	+0.005	+0.000	+0.026	+0.000	+0.000	+0.000	+0.000	+0.000	-0.011	A
B	+0.019	-0.018	+0.000	+0.025	+0.011	+0.011	+0.000	+0.000	+0.019	-0.008	-0.008	+0.000	B
C	+0.015	-0.011	+0.007	-0.009	+0.000	+0.000	+0.043	+0.000	+0.000	+0.000	-0.005	+0.000	C
D	+0.017	+0.000	-0.008	+0.019	+0.016	-0.007	+0.005	+0.009	+0.013	+0.017	-0.008	-0.005	D
E	+0.009	+0.000	+0.010	+0.000	+0.023	+0.000	+0.000	+0.000	+0.000	-0.008	-0.005	+0.000	E
F	+0.000	+0.000	+0.019	+0.013	+0.006	+0.012	-0.006	+0.011	+0.009	+0.000	+0.000	-0.010	F
G	+0.074	+0.000	+0.006	+0.000	+0.010	-0.005	+0.007	+0.000	+0.000	+0.000	-0.014	+0.027	G
H	+0.023	+0.033	+0.010	+0.005	+0.000	+0.000	+0.010	+0.007	+0.005	+0.000	-0.010	-0.008	H

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 #9 Date 10 / 12 / 93 Filter _____ nm
 Operator Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.038	-0.007	+0.008	+0.000	+0.000	+0.010	-0.008	-0.008	+0.000	+0.000	-0.008	+0.000	A
B	+0.007	+0.013	+0.009	+0.017	+0.015	-0.006	-0.006	+0.000	+0.008	-0.010	-0.010	+0.000	B
C	+0.007	+0.019	+0.010	+0.006	+0.021	+0.000	+0.000	+0.000	+0.000	-0.012	-0.010	-0.010	C
D	+0.005	+0.009	+0.019	+0.011	+0.016	+0.000	+0.006	+0.049	+0.006	+0.000	-0.005	+0.000	D
E	+0.000	+0.015	+0.021	+0.033	+0.005	+0.005	+0.005	+0.005	+0.024	-0.011	+0.011	-0.010	E
F	+0.062	+0.023	+0.031	+0.046	+0.017	+0.045	+0.055	+0.000	+0.000	-0.008	-0.006	+0.000	F
G	+0.051	+0.016	+0.016	+0.021	+0.013	+0.013	+0.000	+0.008	+0.013	+0.000	-0.010	+0.008	G
H	+0.008	+0.000	+0.022	+0.018	+0.007	+0.043	+0.026	+0.011	+0.018	+0.005	+0.020	+0.000	H

1 2 3 4 5 6 7 8 9 10 11 12

EASY BEAM

Plate # N053 #1D Date 10 / 12 / 93 Filter _____ nm
 Operator Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.012	-0.007	+0.000	+0.000	+0.000	-0.007	+0.016	+0.000	-0.006	-0.010	-0.005	+0.017	A
B	+0.000	+0.000	+0.009	+0.016	+0.014	+0.000	+0.031	-0.008	-0.008	+0.000	+0.015	+0.000	B
C	+0.000	+0.027	+0.000	+0.000	+0.016	+0.024	+0.054	-0.005	+0.006	-0.007	+0.007	+0.007	C
D	+0.009	+0.012	+0.000	+0.007	+0.027	+0.010	+0.019	+0.006	-0.017	-0.017	-0.010	-0.005	D
E	+0.030	+0.000	+0.009	+0.006	+0.006	+0.000	+0.034	+0.005	+0.065	-0.015	+0.005	+0.053	E
F	+0.006	+0.000	+0.000	+0.019	+0.019	+0.038	+0.065	+0.000	-0.014	-0.007	+0.000	-0.012	F
G	+0.009	+0.015	-0.010	+0.000	+0.013	-0.009	+0.007	-0.009	+0.008	-0.016	+0.000	+0.000	G
H	+0.000	+0.026	+0.000	+0.000	-0.006	-0.006	+0.019	+0.000	+0.000	-0.009	+0.000	-0.010	H

1 2 3 4 5 6 7 8 9 10 11 12

Testing Positive hybrids for binding to ADP-Sepharose purified hemagglutinines

10/16/93

EASY BEAM

Plate #	1h, NOS	Date	10 / 17 / 93	Filter	492	nm
Operator		Comment				
<i>B1616</i>	1E12 C.S.	2E8 C.S.				
1	10 ² <i>µl</i>	30 ¹ <i>µl</i>	25 ⁴ <i>µl</i>	100 ⁵ <i>µl</i>	50 ⁶ <i>µl</i>	25 ⁷ <i>µl</i>
A	+0.000	+0.000	+0.000	+0.035	+0.007	+0.000
B	+0.000	+OVER	+OVER	+1.913	+1.297	+0.691
C	=====	=====	=====	=====	=====	=====
D	=====	=====	=====	=====	=====	=====
E	=====	=====	=====	=====	=====	=====
F	=====	=====	=====	=====	=====	=====
G	=====	=====	=====	=====	=====	=====
H	=====	=====	=====	=====	=====	=====
	1	2	3	4	5	6
	7	8	9	10	11	12

No MeAb binding!

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